The Drosophila RAD21 cohesin persists at the centromere region in mitosis

The *Drosophila* RAD21 cohesin persists at the centromere region in mitosis

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'Cohesin' is a highly conserved multiprotein complex thought to be the primary effector of sister-chromatid cohesion in all eukaryotes. Cohesin complexes in budding yeast hold sister chromatids together from S phase until anaphase, but in metazoans, cohesin proteins dissociate from chromosomes and redistribute into the whole cell volume during prophase, well before sister chromatids separate (reviewed in [1,2]). Here we address this apparent anomaly by investigating the cell-cycle dynamics of DRAD21, the *Drosophila* orthologue of the *Xenopus* XRAD21 and *Saccharomyces cerevisiae* Scc1p/Mcd1p cohesins [3]. Analysis of DRAD21 in S2 *Drosophila* tissue culture cells and live embryos expressing a DRAD21–green fluorescent protein (GFP) fusion revealed the presence of four distinct subcellular pools of DRAD21: a cytoplasmic pool; a chromosome-associated pool which dissociates from chromatin as chromosomes condense in prophase; a short-lived centrosome-associated pool present during metaphase–anaphase; and a centromere-proximal pool which remains bound to condensed chromosomes, is found along the junction of sister chromatids between kinetochores, and persists until the metaphase–anaphase transition. We conclude that in *Drosophila*, and possibly all metazoans, a minor pool of cohesin remains bound to centromere-proximal chromatin after prophase and maintains sister-chromatid cohesion until the metaphase–anaphase transition.

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Results and discussion

We raised antisera against a bacterially expressed carboxy-terminal fragment of DRAD21 (see Supplementary material) and used affinity-purified antibodies to examine DRAD21 localization in *Drosophila* S2 tissue culture cells by indirect immunofluorescence. DRAD21 localization was found to vary in a cell-cycle dependent pattern and to be unusually sensitive to fixation conditions. We used a cell preparation method that included Triton X-100 to increase chromatin antigen accessibility and reduced cytoplasmic staining. In interphase S2 cells, we observed the majority of DRAD21 to be localized to the nucleus with the exclusion of the nucleolus (Figure 1a). In comparison to interphase nuclei, nuclei in mitotic prophase showed a lower level of nuclear DRAD21 which was distributed along the condensing chromosomes (Figure 1b). During prometaphase, as chromosomes became fully condensed, DRAD21 was restricted to discrete chromosomal regions (Figure 1c). Cells in metaphase show DRAD21 localized to the centromere-proximal regions of fully condensed chromosomes aligned at the metaphase plate (Figure 1d). After sister-chromatid separation in anaphase, chromatin-associated DRAD21 was undetectable (Figure 1e). Lastly, re-localization of DRAD21 to chromatin was seen to commence in telophase cells (Figure 1f). These data provide clear evidence that, although the majority of DRAD21 disappears from chromosomes during prophase, a sub-pool remains associated with centromere-proximal chromatin until sister-chromatid separation, then rapidly disappears in early anaphase.

To analyse the centromere-proximal pool of DRAD21 in more detail we treated cells with colchicine to disaggregate chromosomes from the metaphase plate. Cells simultaneously stained for DNA with DAPI (Figure 2a), for DRAD21 with affinity-purified antibody (Figure 2b) and with the MPM2 monoclonal antibody (Figure 2c) were examined. In *Drosophila* cells, MPM2 recognises mitotic phosphoepitopes that predominantly localise to the kinetochore and centrosome [4]. Merged images (Figure 2d,e) clearly show chromosomally localized DRAD21 restricted to centromere-proximal heterochromatin, which remains joined in standard cytological preparations of *Drosophila* metaphase cells. This metaphase pool of DRAD21 is only found along the junction of paired sister chromatids and in between and perpendicular to the kinetochores. These data show that DRAD21 persists on chromosomes in metaphase and is appropriately located to mediate sister-chromatid cohesion up until the metaphase–anaphase transition.

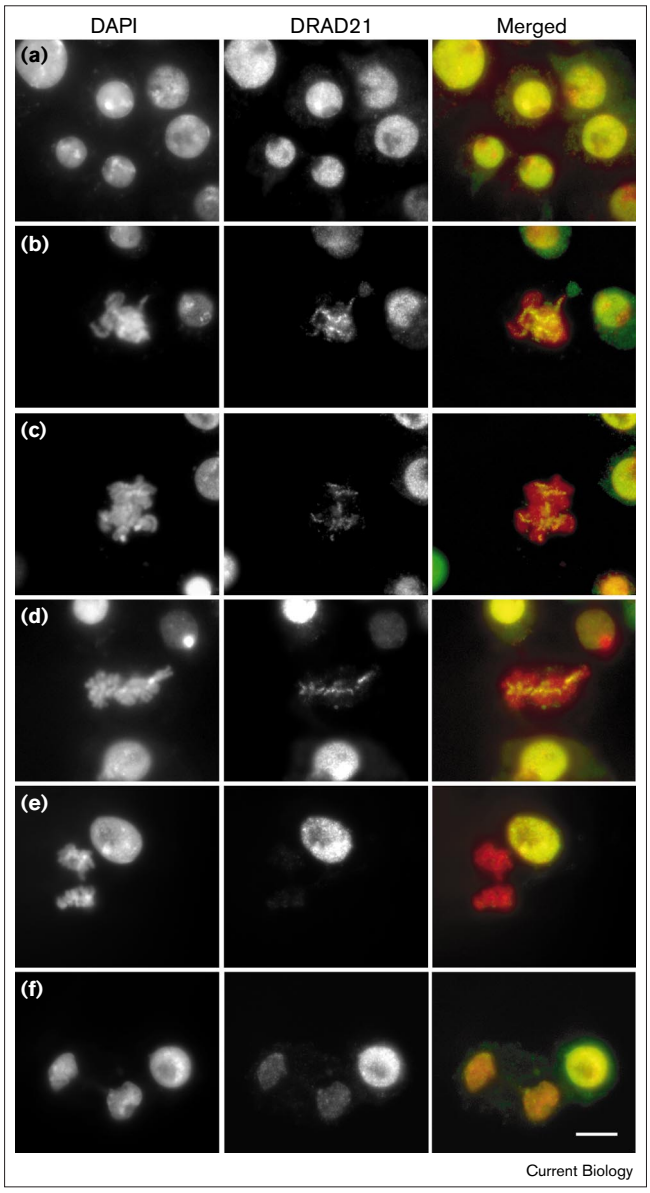


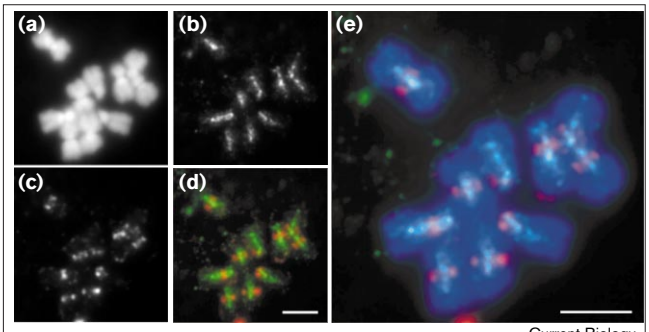
Figure 1

Cell-cycle localisation of DRAD21 in S2 *Drosophila* cells. Cells were immunostained to reveal DRAD21 and counterstained for DNA with DAPI. In the merged images DRAD21 is in green and DNA in red. **(a)** Interphase cell showing strong nuclear staining. **(b)** Prophase cell in early stages of chromosome condensation showing restriction of DRAD21 to the condensing chromosomes. **(c)** Prometaphase cell with condensed chromosomes and DRAD21 staining in discrete chromosomal regions. **(d)** Late prometaphase cell with chromosomes congressed at the metaphase plate. At this stage DRAD21 is highly restricted to the region of the chromosomes aligned at the equator of the spindle. **(e)** Anaphase cell showing significant reduction of DRAD21 staining following sister-chromatid separation. **(f)** Telophase cell showing faint chromosomal DRAD21 staining. A faint cytoplasmic signal was observed in all cells irrespective of cell-cycle stage when cells were not Triton-extracted (data not shown). The scale bar represents 5 μm.

As a complement to the immunolocalization studies described above, we investigated the cell-cycle dynamics of a DRAD21–GFP fusion in living cells. Expression of DRAD21–GFP had no detrimental affect on development and adults were fully viable and fertile. Time-lapse observation of a cycle 13 syncytial embryo (Figure 3a, and Supplementary material) revealed DRAD21–GFP to be predominantly nuclear in interphase nuclei (0 min). Nuclei at the anterior and posterior poles of embryos at this stage commence mitosis just before those in the mid-region. As nuclei prepared to divide, DRAD21–GFP started to dissociate from chromosomes and a cytoplasmic cloud of fluorescence formed (3–6 minutes). Close examination revealed the appearance of small brightly fluorescent foci between the nuclei (6 minutes). Telophase nuclei nearest the poles of the embryo showed nuclear DRAD21–GFP fluorescence (9 minutes) followed shortly by nuclei in the middle of the embryo (12 minutes). By 15 minutes, all the nuclei were again in interphase, the bright foci were no longer evident and DRAD21–GFP was again mostly nuclear. Time-lapse observations of DRAD21–GFP dynamics in the asynchronous post-cellularization embryonic divisions (data not shown) were essentially the same as shown in Figure 3a. As the lag between *de novo* GFP translation and the formation of a fluorescent GFP chromophore is of the order of 40 minutes in *Drosophila* embryos [5], the observation of nuclear GFP fluorescence commencing in telophase indicates that most DRAD21–GFP molecules are not proteolysed at the end of each embryonic nuclear division.

To follow more closely the intracellular dynamics of DRAD21–GFP, we microinjected rhodamine-labelled tubulin into embryos expressing DRAD21–GFP and examined syncytial mitoses at higher magnification. Figure 3b (and Supplementary material) shows a cycle 11 embryo undergoing a synchronous mitosis. In interphase (0 minutes), DRAD21–GFP was mostly nuclear and rhodamine–tubulin remained excluded from the nucleus. As prophase commenced (2 minutes), DRAD21–GFP began to dissociate from chromatin. When spindles became fully formed in metaphase (4 minutes), DRAD21–GFP was largely cytoplasmic, although some residual chromosomal and/or microtubule-associated DRAD21–GFP remained. Brightly fluorescent foci which colocalised with centrosomes were consistently observed on all high-magnification images of DRAD21–GFP-expressing embryos in metaphase. By anaphase (6 minutes), centrosome fluorescence was slightly more intense and chromosome and microtubule fluorescence diminished. At telophase (8 minutes) the centrosomal foci were no longer visible and by interphase (10 minutes), the DRAD21–GFP was again mostly nuclear. When we fixed DRAD21–GFP-expressing embryos we detected weak microtubule and chromosomal GFP fluorescence at metaphase which was diminished in anaphase (see Supplementary material). Centrosomal

Figure 2



Metaphase chromosome localisation of DRAD21. **(a)** Cells stained for DNA with DAPI, **(b)** immunostained with antibodies against DRAD21 and **(c)** immunostained with antibodies against MPM2. **(d)** Merged image showing DRAD21 (green) and MPM2 (red). **(e)** Merged image showing DNA staining (blue) MPM2 (red) and DRAD21 (green). The MPM2 antibodies strongly label sister kinetochores of each chromosome and DRAD21 is concentrated along the junction of paired sister chromatids between the kinetochores. The scale bar represents 5 μm.

GFP fluorescence was, however, not detected, indicating that this pool of DRAD21 is sensitive to fixation.

Taken together, our imunolocalization and live GFP analyses have identified four distinct pools of DRAD21 in *Drosophila* cells. One is cytoplasmic, the second is chromosome-associated and dissociates in prophase, the third is a short-lived centrosome-associated pool present during metaphase–anaphase, and the fourth is a centromere-proximal pool which persists on chromosomes until the metaphase–anaphase transition. The overall similarity in cell-cycle dynamics determined by immunofluorescence and live GFP fusion studies strongly suggests that our observations reflect the true dynamics of the endogenous DRAD21 protein in diploid *Drosophila* cells. Some minor differences were observed in the two experimental systems, probably attributable to differences between embryonic syncytial and cultured cell divisions, protein redistribution upon fixation and/or DRAD21–GFP overexpression. Difficulties in detecting DRAD21 in embryos with our antibody and the lack of a *Drad21* mutant has prevented us from testing these possibilities or confirming that the DRAD21–GFP fusion is functional. However, the strong correlation between cell-cycle dynamics of DRAD21–GFP and the endogenous DRAD21, and the absence of any deleterious phenotype associated with DRAD21–GFP expression, strongly suggests that it can be recruited into functional cohesin complexes.

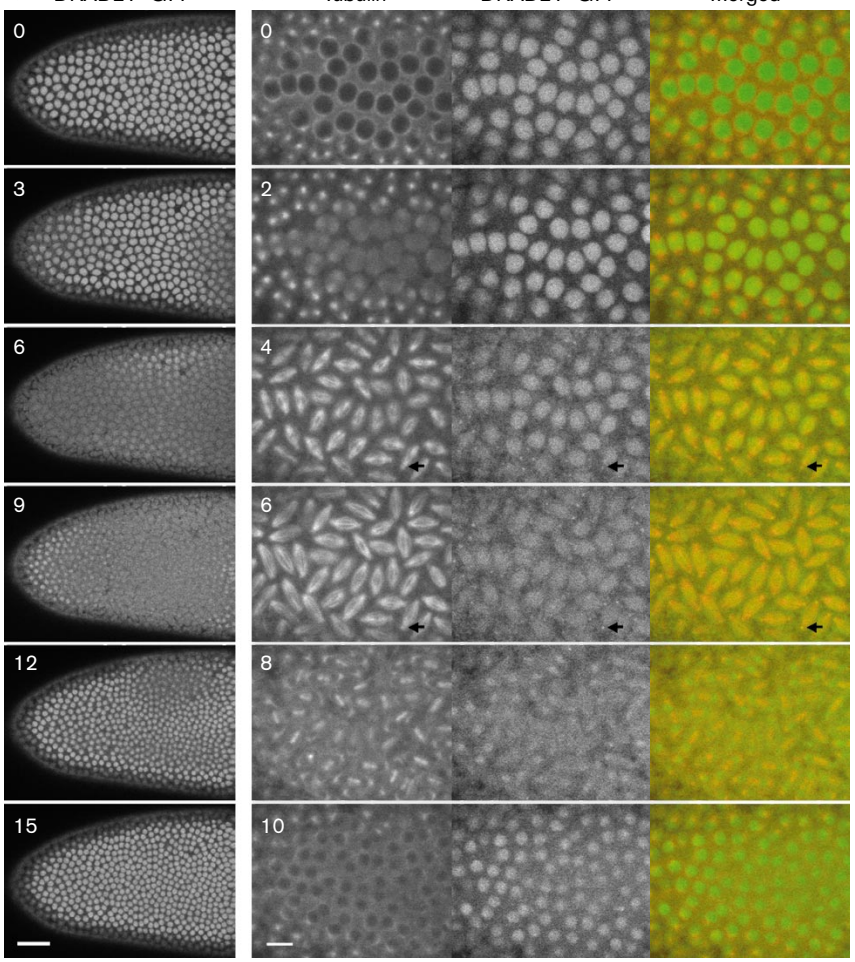
As the primary role of the cohesin complex is to link chromatids and control the timing of sister-chromatid separation, we can only speculate at this point why DRAD21 associates transiently with centrosomes during metaphase–anaphase. Given its transient nature during a time when the *S. cerevisiae* orthologue of DRAD21 is being degraded, we speculate that we are observing an overloading of the proteasome degradation pathway, causing transient aggresome formation. In mammalian cells, protein overexpression or inhibition of the anaphase-promoting complex (APC/C) has been shown to cause accumulation of intracellular protein aggregates, called 'aggresomes', which form at the microtubule-organising center/centrosome region of cells [6,7]. In *Drosophila*, APC/C has been shown to be associated with both spindle microtubles and centrosomes, and cyclin B, which is also targeted for degradation at metaphase–anaphase, has been observed to associate transiently with centrosomes immediately before its degradation [8]. Overloading of the proteasome and consequent aggresome formation would also be consistent with accumulation of DRAD21–GFP on spindle microtubules and persistence of low levels of DRAD21–GFP on anaphase chromosomes (Figure 3b, and Supplementary material). However appealing this hypothesis, we cannot rule out the possibility that DRAD21 may have an active role in spindle dynamics or checkpoint monitoring and that its transient association with the centrosome is a reflection of this.

Our analysis of DRAD21 intracellular dynamics extend previous studies of metazoan cohesin complexes by clearly showing that a small percentage of the total cell content of the cohesin subunit RAD21 can be detected on condensed chromosomes at metaphase. These molecules are restricted to where sister chromatids are in contact, suggesting that they are actively participating in the physical maintenance of sister-chromatid cohesion until the metaphase–anaphase transition. Our data are supported by the recent study of the *Xenopus* XSA1 cohesin subunit [9]. Using XSA1-specific antibodies, Losada *et al.* [9] showed weak labelling of mitotic chromatin reassembled from *Xenopus* egg extracts, but were unable to detect XSA1 or any other cohesin subunit on intact metaphase chromosomes. Our data provide strong evidence that cohesin regulates the dissolution of sister-chromatid cohesion in higher eukaryotes via a two-step mechanism, the first step occurring in prophase, to facilitate chromosome condensation, and the second at metaphase–anaphase to separate sister chromatids. As *Drosophila* and vertebrate homologues of Esp1p, the *S. cerevisiae* 'separin' protein essential for Scc1p cleavage, have been identified [10,11] it seems likely that the maintenance of sister-chromatid cohesion in metaphase by centromere-proximal cohesin and its dissolution by proteolysis at the onset of anaphase occurs in all metazoans.

Supplementary material

Supplementary material including DRAD21 expression, localization of DRAD21–GFP in fixed embryos, time-lapse movies and additional methodological detail is available at http://current-biology.com/supmat/supmatin.htm.

Figure 3



Time-lapse confocal fluorescence micrographs of DRAD21–GFP in living syncytial embryos. **(a)** Low-magnification time-series of the anterior end of a cycle-13 embryo as it undergoes a quasi-synchronous mitosis. Time in minutes is shown in the upper left corner of each image. The scale bar represents 25 μm. **(b)** High-magnification time-lapse images of a rhodamine–tubulin-injected embryo expressing DRAD21–GFP. Tubulin is shown in the left, DRAD21–GFP in the centre and the merged image on the right with tubulin in red and DRAD21 in green. The scale bar represents 10 μm. See text for full description and Supplementary material for time-lapse movies of these data.

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References

1. Dej KJ, Orr-Weaver T: **Separation anxiety at the centromere.** *Trends Cell Biol* 2000 10:392-399.
2. Nasmyth K, Peters J-M, Uhlmann F: **Splitting the chromosome: Cutting the ties that bind sister chromatids.** *Science* 2000, **288:**1379-1384.
3. Warren WD, Lin E, Nheu T, Hime GR, McKay MJ: ***Drad21*, a *Drosophila* rad21 homologue expressed in S phase mitotic cells.** *Gene* 2000, **250:**77-84.
4. Logarinho E, Sunkel C: **The Drosophila POLO kinase localises to multiple compartments of the mitotic apparatus and is required for the phosphoryation of MPM2 reactive epitopes.** *J Cell Sci* 1998, **111:**2897-2909.
5. Hazelrigg T, Liu N, Hong Y, Wang S: **GFP expression in *Drosophila* tissues — time requirements for formation of a fluorescent product.** *Dev Biol* 1998, **199:**245-249.
6. Johnston J, Ward C, Kopito R: **Aggresomes: a cellular response to misfolded proteins.** *J Cell Biol* 1998, **143:**1883-1898.
7. Garcia-Mata R, Bebök A, Sorscher E, Sztul E: **Characterization of dynamics of aggresome formation by a cytosolic GFP-chimera.** *J Cell Biol* 1999, **146:**1239-1254.
8. Huang J, Raff JW: **The disappearance of cyclin B at the end of mitosis is regulated spatially in *Drosophila* cells.** *EMBO J* 1999, **18:**2184-2195.
9. Losada A, Yokochi T, Kobayashi R, Hirano T: **Identification and characterization of SA/Scc3p subunits in the *Xenopus* and human cohesin complexes.** *J Cell Biol* 2000, **150:**405-416.
10. Zou H, McGarry TJ, Bernal TK, Kirschner MW: **Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis.** *Science* 1999, **285:**418-422.
11. Rubin G, Yandell M, Wortman J, Miklos G, Nelson C, Hariharan I, *et al.*: **Comparative genomics of the eukaryotes.** *Science* 2000, **287:**2204-2215.